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A STUDY OF THE POTENTIAL WAYS IN WHICH OZONE COULD REDUCE ROOT GROWTH AND NODULATION OF SOYBEAN*

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Abstract—The possible mechanisms by which ozone reduces root growth and nodulation of soybean were investigated. Ozone did not appreciably penetrate the plant growth substrates nor did it oxidize soil organic matter to form compounds inhibitory to *Rhizobium*. When ozone was excluded from the plant foliage, but not from the soil, root growth and nodulation were not reduced. However, when plant tops were directly exposed to ozone, root growth and nodulation were reduced. These results indicated that observed reductions in root growth and nodulation did not occur by way of the soil, but resulted from an effect of ozone on the plant foliage.

INTRODUCTION

Acute and chronic ozone exposures induce foliar injury, suppress nodulation, and inhibit root growth more than top growth in legumes (Engle and Gabelman, 1967; Manning *et al.*, 1971; Tingey and Blum, 1973; Tingey *et al.*, 1973b). Ozone could suppress root growth and nodulation either directly by a) diffusing into the soil to inhibit growth and nodulation, b) by oxidizing soil organic matter to form toxins, c) or indirectly by altering foliar metabolism and reducing the quality and/or quantity of photosynthate translocated to the roots. The objective of this study was to determine which mechanism or combination of mechanisms could explain ozone suppression of root growth and nodulation.

METHODS AND MATERIALS

Ozone penetration into plant growth substrates

Cylindrical chambers each consisting of a soil holding area and an air sampling area separated by a perforated plate were constructed. The soil holding area was constructed of 2 × 14.6 cm (14 cm i.d.) Plexiglas rings stacked on top of one another and held in place with bolts (outside of the rings) through top and base plates (20 × 20 cm). The top plate had a single circular opening (154 cm^2). The air sampling area was located between the base plate and a perforated plate at the bottom of the soil handling area. The air sampling area consisted of a solid base plate to which a 2 × 14.6 cm dia. ring topped with a perforated plate containing 28 holes (0.4 cm dia.) was fixed. Air was sampled from this area through a 0.63 cm ID sampling port and the ozone content of the air exiting the base of the chambers was measured using the alkaline potassium iodine method (U.S. Department of Health, Education, and Welfare, 1965). The depth of substrates was varied in 2 cm increments for each study by changing the number of rings in the soil holding area. Before the 2 h ozone exposure, the outsides of the chambers were covered with plastic film and aluminum foil. Air containing either 0 or 0.5 ppm ozone was drawn through the soil columns at a linear velocity of 12.7 cm min^{-1}. The penetration of ozone into several substrates was studied.

The substrates and their mean water content (no water was added to substrate) over the experimental periods were: gravel (3.7%), sand (1.8%), Jiffy mix† (27.1%), and Jiffy mix + gravel (1:2:v:v) (6.1%). Conclusions were based on 3 replicates per substrate per ozone concentration.

The diffusion of ozone into soil substrate was measured by the oxidation of dichlorophenol–indophenol. The Jiffy mix + gravel substrate was saturated with a solution of dichlorophenol–indophenol (1.7 mM) and allowed to drain overnight yielding a mean water content of 19%. The dye coated substrate was exposed to either 0 or 0.5 ppm ozone for 1, 2, or 4 hr. Immediately after exposure, 1 cm layers of substrate were removed; the dye was extracted with water and the amount of unoxidized dye was measured spectrophotometrically at 600 nm.

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† Jiffy mix is a trade mark of a product containing peat moss and vermiculite. Mention of a trade mark or proprietary product by North Carolina State University, the Environmental Protection Agency does not imply approval to the exclusion of other products that may be suitable.

Ozone and the production of inhibitors in substrates

Jiffy mix + gravel in 1800 cm^3 (10 cm dia.) plastic pots was exposed in two different experiments to either 0 and 0.5 ppm ozone for 4 hr or to 0 or 0.18 ppm ozone for 8 hr/day for 30 days. Water was added only to the 30 day experiment. Mean water content for the 4 hr experiment was 8.7% and 18% for the 30 day experiment. Following the completion of exposure, the upper 2 cm of substrate were removed from each of 4 pots and combined into a single sample. A 25 g aliquot was extracted for 24 hr with acetone in a Soxhlet extractor. The extracts were concentrated by flash evaporation and adjusted to a final volume (5 ml) with acetone. The concentrated extracts were incorporated in sensitivity discs (Difco Laboratories, 1953) and tested for toxicity to *Rhizobium japonicum* (5–7 day cultures*, $3I_1b$ 59 NC 1946) innoculated on yeast mannitol agar. Zones of inhibition around the sensitivity discs were measured daily for 4 days. Conclusions were based on 4 replicates of 4 discs per ozone treatment. Water extracts were also obtained from both studies and tested for toxicity.

Ozone effects on plant growth and nodulation

Soybean (*Glycine max* (L.) Merr.) cv'Dare'seeds (5/pot) were planted in Jiffy mix + gravel in 10 cm diam. pots and innoculated with 20 ml of yeast mannitol broth containing 5–7 day old cultures of *Rhizobium japonicum* ($3I_1b$ 59 NC 1946) and covered with 1–2 cm of substrate. The plants were grown in the Southeastern Plant Environmental Laboratories as previously described (Kramer *et al.*, 1970; Tingey *et al.*, 1973a). Plants were watered twice daily—once with a complete nutrient solution and once with distilled water (Raper and Johnson, 1971). One week after planting the seedlings were thinned to one plant per pot. Two weeks after seeding the plants were divided into 2 equal groups. The foliage of 1 group was enclosed in plastic bags and the foliage of the other group was not enclosed. Both bagged and unbagged plants were exposed to ozone as previously described (Heck *et al.*, 1968; Tingey *et al.*, 1973a). Immediately following exposure, the plastic bags were removed and all plants were returned to the growth environment.

Plants were harvested, separated into tops and roots 4, 8, and 16 days following exposure and the dry weights were measured.

Data analysis

Top and root relative growth rates were calculated as described by Radford (1967). Significant differences among treatments were determined using analysis of variance procedures.

* Culture ($3I_1b$ 59 NC 1946) was obtained from B. E. Caldwell Soybean Investigations, Plant Industry Station, Beltsville, MD. 20705.

RESULTS AND DISCUSSION

Ozone penetration into plant growth substrates

When a total of 120 μg ozone (0.5 ppm for 2 hr) was applied to either 2 or 4 cm thick columns of gravel, sand, Jiffy mix, or Jiffy mix + gravel, no ozone was detected in the air exiting the columns. Ozone diffusion into Jiffy mix + gravel as measured by the oxidation of dichlorophenol–indophenol, although not significant, occurred in the first cm of substrate after 2 and 4 hr of treatment (Table 1). These results suggest that ozone does not penetrate into the substrates used to any appreciable extent. This is consistent with the reports that ozone is unstable in the presence of moisture, organic matter or rough surfaces and supports the concept that the soil is an ozone sink (Alder and Hill, 1950; Bohn, 1972; MacDowell, 1974; Turner *et al.*, 1973).

Ozone and the production of inhibitors in substrates

Ozone oxidizing the organic portion of the substrate could form substances inhibitory to *Rhizobium*. In the sensitivity disc assays of the acetone and water extracts from substrates exposed for a short time (0 or 0.5 ppm for 4 hr) or for a long time (0 or 0.18 ppm for 8 hr/day for 30 days), the zones of inhibition were not significantly different from the control. This suggests, at least for the substrates tested, that ozone does not induce the formation of substances inhibitory to *Rhizobium*.

Ozone effects on plant growth and nodulation

The relative growth rates of the plant tops and roots and nodulation (increase in nodule number) of the ozone exposed plants were significantly less than either of the controls or the ozone + bag treatment (Table 2). This supports the previous reports that ozone suppresses top and root growth and nodulation (Engle and Gabelman, 1967; Manning *et al.*, 1971; Tingey and Blum, 1973; Tingey *et al.*, 1973b). The lack of significant differences between the ozone + bag (where ozone was excluded from the foliage) and the two types of controls supports the concept that

Table 1. Depth of ozone diffusion into Jiffy mix + gravel*†

Duration of treatment (hr)	Unoxidized dichlorophenol–indophenol recovered from soil after treatment expressed as a % of control (depth in cm) 0–1	1–2	2–4
1	100 ± 10‡	100 ± 14	104 ± 13
2	88 ± 6	107 ± 7	94 ± 8
4	84 ± 11	91 ± 10	100 ± 12

* No statistically significant differences were found at the 5% significance level.

† Substrate was subjected to charcoal filtered air (control) or 0.5 ppm of ozone. Each mean is an average of 4 observations.

‡ Standard error of the mean.

Table 2. The effect of ozone on the growth and nodulation of bagged and unbagged soybean plants*

Treatment	Relative growth rate (days^{-1}) Top growth	Root growth	Increase in nodule numbers (nodules days^{-1})
Control	0.11	0.09	6.8
Control + bag	0.11	0.10	7.4
Ozone	0.08	0.07	2.7
Ozone + bag	0.11	0.10	6.1

* Two week old soybean plants were exposed to 0 or 0.5 ppm of ozone for 4 hr. During exposure half of the tops were enclosed in plastic bags. Plants were harvested at 4, 8, and 16 days after exposure. Each value was derived from 9 observations. The ozone treatment was significantly lower than the other 3 treatments at the 0.05 significance level.

ozone does not penetrate the soil to cause a direct effect on root growth or nodulation. Rather, the reductions in root growth and nodulation resulted indirectly from ozone induced alterations in foliar metabolism and translocation.

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